

WHAT IS CLAIMED IS:

1. An expression cassette useful for the secretion of a heterologous protein from insect cells as a fusion protein comprising a polynucleotide encoding from its 5' to 3' direction:

- 5 a) a promoter;
- b) a signal peptide;
- c) an insect cell secretion competent polypeptide; and
- d) a heterologous protein wherein the polynucleotide sequences encoding (b)
- (c) and (d) are linked in frame and wherein the insect cell secretion
- 10 competent polypeptide is not an immunoglobulin Fc region.

2. The expression cassette of Claim 1 wherein the promoter sequence is selected from the group consisting of a viral promoter sequence, an insect cellular promoter sequence or a mammalian promoter sequence.

15 3. The expression cassette of Claim 1 further comprising a polynucleotide sequence encoding an enhancer functionally linked to the promoter.

4. The expression cassette of Claim 3, wherein the enhancer is a viral enhancer.

20 5. The expression cassette of Claim 1 wherein the sequence encoding the secretion competent polypeptide sequence is linked in frame to the sequence encoding the heterologous protein by a sequence encoding a linker peptide.

6. The expression cassette of Claim 1 wherein the secretion competent polypeptide is selected from the group consisting of insect juvenile hormone

10083590-0220

esterase, human granulocyte macrophage colony stimulating factor, human interleukin-4, mouse interleukin-4, tissue plasminogen activator, transferrin, gamma interferon, transforming growth factor beta, epidermal growth factor, insect adipokinetic hormone precursor, insulin-like growth factor 1, stem cell factor, leptin, human growth hormone, erythropoietin, interleukin-5, interleukin-6, tumor necrosis factor alpha, tissue inhibitor of metalloproteases-1, secreted alkaline phosphatase, soluble isoforms of the alpha subunit of the granulocyte macrophage colony stimulating factor receptor, and soluble isoforms of the beta subunit of the granulocyte macrophage colony stimulating factor receptor.

7. The expression cassette of Claim 6 wherein the secretion competent polypeptide is selected from the group consisting of insect juvenile hormone esterase and human granulocyte macrophage colony stimulating factor.

8. A vector useful for the secretion of a heterologous protein from eukaryotic cells comprising an expression cassette comprising a polynucleotide encoding from its 5' to 3' direction:

- a) a promoter;
- b) a signal peptide;
- c) an insect cell secretion competent polypeptide; and
- d) a heterologous protein wherein the polynucleotide sequences encoding (b) (c) and (d) are linked in frame and wherein the insect cell secretion competent polypeptide is not an immunoglobulin Fc region.

9. The vector of Claim 8 wherein the promoter is selected from the group consisting of a viral promoter, an insect cellular promoter or a mammalian promoter.

10. The vector of Claim 8 further comprising a DNA sequence encoding an enhancer functionally linked to the promoter.

11. The vector of Claim 10, wherein the enhancer is a viral enhancer.

12. The vector of Claim 8, further comprising a selectable marker gene.

5 13. An insect cell transformed with the expression cassette of Claim 1.

14. The cell of Claim 13 wherein the insect cell is from *Bombyx mori*.

15. A method of secreting a heterologous protein, comprising introducing into an insect cell an expression cassette comprising a polynucleotide encoding from its 5' to 3' direction:

10 a) a promoter;

b) a signal peptide;

c) an insect cell secretion competent polypeptide; and

d) a heterologous protein wherein the polynucleotide sequences encoding (b)

(c) and (d) are linked in frame under conditions wherein the heterologous 15 protein is expressed and secreted from the insect cell.

16. The method of Claim 15 wherein the promoter is selected from the group consisting of a viral promoter, an insect cellular promoter or a mammalian promoter.

20 17. The method of Claim 15 wherein the expression cassette further comprises a DNA sequence encoding an enhancer functionally linked to the promoter.

1008350-02720

18. The method of Claim 17, wherein the enhancer is a viral enhancer.

19. The method of Claim 15 wherein the sequence encoding the secretion competent polypeptide sequence is linked in frame to the sequence encoding the heterologous protein by a sequence encoding a linker peptide.

5 20. The method of Claim 15 wherein the secretion competent polypeptide is selected from the group consisting of insect juvenile hormone esterase, human granulocyte macrophage colony stimulating factor, human interleukin-4, mouse interleukin-4, tissue plasminogen activator, transferrin, gamma interferon, transforming growth factor beta, epidermal growth factor, insect adipokinetic hormone precursor, insulin-like growth factor 1, stem cell factor, leptin, human growth hormone, erythropoietin, interleukin-5, interleukin-6, tumor necrosis factor alpha, tissue inhibitor of metalloproteases-1, secreted alkaline phosphatase, soluble isoforms of the alpha subunit of the granulocyte macrophage colony stimulating factor receptor, and soluble isoforms of the beta subunit of the granulocyte macrophage colony stimulating factor receptor.

10 21. The method of Claim 20 wherein the secretion competent polypeptide is selected from the group consisting of insect juvenile hormone esterase and human granulocyte macrophage colony stimulating factor.

15 22. A method of secreting a heterologous protein from mammalian cells, comprising introducing into an mammalian cell an expression cassette comprising a polynucleotide encoding from its 5' to 3' direction: a) a promoter b) a signal peptide; c) a secretion competent polypeptide selected from the group consisting of juvenile hormone esterase or human granulocyte macrophage colony stimulating factor; and d) a heterologous

10083500 022702

PATENT

-39- Attorney Docket No. 028722-207

protein wherein the polynucleotide sequences encoding (b) (c) and (d) are linked in frame under conditions wherein the heterologous protein is expressed and secreted from the mammalian cell.

10083590-022702